Circulating resistin in lean, obese, and insulin-resistant mouse models: lack of association with insulinemia and glycemia

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Resistin is a 12.5-kda cysteine-rich protein secreted by adipocytes, which has been proposed to link obesity with insulin resistance and diabetes (14, 17, 38). Initial studies in mice suggested that resistin induces insulin resistance, but subsequent animal studies revealed conflicting data regarding whether resistin expression is increased (38, 39) or decreased (16, 19, 25, 26, 43) in obesity, and decreased (12, 27, 36, 38, 39) or increased (8, 43) by thiazolidinediones (TZDs), drugs known to improve insulin resistance. Moreover, although serum resistin levels measured by Western blot appeared to be elevated in genetic (ob/ob and db/db) and diet-induced models of obesity and decreased after TZD treatment or fasting (38), only a limited number of mice were studied, and thus no statistical evaluation of this difference could be performed. Furthermore, data from another study showed decreased serum resistin levels in obese mice by use of an ELISA with low accuracy and sensitivity (22). Thus the relevance and physiological role of circulating resistin in mice remain to be elucidated. The inconsistencies between animal studies with regard to mRNA expression in adipose tissue or function of resistin may be related to methodological limitations and/or biological variation between adipose mRNA expression levels and serum concentrations (40). There are also in vitro studies supporting the presence of resistin in human primary monocyte-derived macrophages (29), which, in conjunction with differences in regulation of resistin clearance, could contribute to the inconsistencies observed between adipose mRNA expression and serum levels of resistin.

Although adipocyte resistin mRNA expression and circulating levels may theoretically be discordant due to a potential regulation at both the transcriptional and posttranscriptional levels, as well as contribution to circulation by tissues other than adipocytes, the concomitant evaluation of both mRNA expression and serum levels has been limited by the lack of specific resistin assays. Moreover, the regulation of resistin mRNA and serum levels by physiological and pharmacological factors, such as the weight-reducing/insulin-sensitizing drugs MTII and CNTFAx15, remains to be elucidated. We have thus conducted studies in four different mouse models of adiposity and insulin resistance, including lean as well as diet-induced obese (DIO) C57BL/6J, high fat-fed TNF-α/−/− mice, and brown adipose tissue (BAT)-deficient uncoupling protein-diphtheria toxin A chain (UCP1-DTA) mice. We also studied whether treatment with the weight-reducing and insulin-sensitizing compound, MTII, an α-melanocyte-stimulating hormone analog, or CNTFAx15, a ciliary neurotrophic factor analog, alters resistin mRNA expression and/or circulating levels in lean and DIO C57BL/6J mice. We find that resistin mRNA expression is similar in DIO and lean C57BL/6J mice, as well as in TNF-α/−/− and wild-type (WT) mice. Circulating resistin levels, however, are higher in DIO C57BL/6J, high fat-fed TNF-α/−/−, and UCP1-DTA mice compared with lean controls. Moreover, although resistin mRNA expression is upregulated by MTII treatment for 24 h and downregulated by CNTFAx15 treatment for 3 or 7 days, circulating resistin levels are not altered by MTII or CNTFAx15 treatment. In addition, serum resistin levels, but not resistin mRNA expression levels, are correlated with body weight, and neither resistin mRNA expression nor serum resistin levels are correlated with serum insulin or glucose levels. We conclude that transcriptional regulation of resistin in WT does not correlate with circulating resistin levels and that circulating resistin is unlikely to play a major endocrine role in insulin resistance or glycemia in mice.

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Innovative Methodology

MATERIALS AND METHODS

Animals

Three- to five-week-old C57BL/6J and 6-wk-old TNF-α−/− (B6/29SF2/J) and WT (B6/29SC) mice were obtained from Jackson Laboratories (Bar Harbor, ME). Male UC1-P1-DTA mice (6–8 wk old; FVB background) were obtained from a colony maintained at the Beth Israel Deaconess Medical Center. All animals were allotted a 7-day acclimation period with access to water ad libitum and were maintained at 25°C with a 12:12-h light-dark cycle (lights on from 0630 to 1830) for the duration of the study. During the acclimation period, mice were handled and weighed daily to ensure stable body weight and sufficient acclimation to daily handling before the onset of experiments. A normal chow or high-fat diet, as previously described (3), was fed to respective mice, as outlined below.

All animals were handled in accordance with the principles and guidelines established by the National Institutes of Health. The protocol was approved by the Institutional Review Board at Beth Israel Deaconess Medical Center, Boston, MA.

Experimental Procedures

Four separate cohorts of animals were used to obtain the data presented herein. Three cohorts were used to explore changes in resistin WAT mRNA expression and serum resistin levels, as well as alterations in serum insulin and glucose levels, in lean and/or DIO C57BL/6J mice treated with short-term (MTII) or long-term (CNTFAx15) drug treatment and in TNF-α−/− mice compared with respective WT controls. The fourth cohort was used to explore serum resistin levels in obese BAT-deficient UC1-P1-DTA mice. All animals were euthanized by CO2 asphyxiation at the times indicated in the specific experiments below, followed by immediate collection of sera by intracardiac puncture and collection of WAT, both of which were stored at −80°C, as previously described (24).

Experiment 1: evaluation of resistin WAT mRNA expression and serum levels in lean and DIO C57BL/6J mice treated with MTII for 24 h. One subcohort of lean (chow fed) and DIO (high-fat fed for 18 wk) C57BL/6J mice (n = 6/group) were administered intraperitoneal injections of MTII (100 µg qid) for 24 h. A second subcohort of lean C57BL/6J mice (n = 8/group) were administered three different doses of MTII (5, 25, and 100 µg qid ip) for 24 h. Control groups (PBS treated, and pair fed to the MTII-treated group for the 1st cohort, PBS treated only for the 2nd cohort) were also included and were maintained on the same diet as their corresponding treatment group (n = 6 or 8/group for each respective cohort), as previously described (3). Pair feeding was administered with a 1-day lag behind the corresponding-treated group, as previously described (3). Body weights were measured between 0800 and 1000 at the beginning and the end of the study with an analytic balance, as previously described (24, 30).

Experiment 2: evaluation of resistin WAT mRNA expression and serum levels in DIO C57BL/6J mice treated with CNTFAx15 for 3 and 7 days. One cohort of DIO (high-fat fed for 18 wk) C57BL/6J mice was administered subcutaneous injections of CNTFAx15 once daily for 3 days at either 0.1 or 1.0 µg·g−1·day−1 (n = 10/group) and euthanized 4 days after the last injection. A second cohort of DIO C57BL/6J mice was administered subcutaneous injections of CNTFAx15 once daily for 7 days at either 0.1 or 0.3 µg·g−1·day−1, and the two subcohorts of this cohort of mice were euthanized either 24 h or 4 days after the last injection (n = 5/group). Control groups (PBS treated and pair fed to the respective high dose CNTFAx15-treated group) were also included (n = 10 or 5/group for each respective cohort). Pair feeding was administered with a 1-day lag behind the corresponding-treated group, as previously described (3). Body weights were measured between 0800 and 1000, as described above.

Experiment 3: evaluation of resistin WAT mRNA expression and serum levels in TNF-α−/− (B6/29SF2/J) compared with respective WT (B6/29SC) mice. Eleven-week-old WT (B6/29SC) and TNF-α−/− (B6/29SF2/J) mice (n = 6/group) were euthanized after 5 wk of high-fat feeding. Body weights were measured weekly between 1430 and 1630 with an analytic balance, as described above.

Experiment 4: evaluation of serum resistin levels in obese BAT-deficient UC1-P1-DTA mice. UC1-P1-DTA mice (n = 12) were killed after 14 wk of chow feeding. Body weights were measured between 0800 and 1000, as described above.

Quantitative PCR Analysis of Resistin mRNA Expression in WAT

Resistin mRNA expression in WAT was assessed and quantified using real-time quantitative PCR (RT-PCR). Total RNA was purified using the RNA STAT-60 Total RNA/mRNA isolation reagent according to the manufacturer’s instructions (Tel-Test, Friendswood, TX). Quantity and purity were measured by ultraviolet absorbance at 260 and 280 nm. Total RNA (1 µg) was reverse transcribed (Programmable Thermal Controller, PTC-100, MJ Research) using the Advantage RT-for-PCR kit according to the manufacturer’s instructions (Clontech Laboratories, Palo Alto, CA). Real-time quantitative PCR reactions were performed in an automated Stratagene MX4000 Multiplex QPCR System (Stratagene, LaJolla, CA) using SYBR Green master mix (Applied Biosystems, Foster City, CA). Primers had been previously tested for specificity using control reactions without adding reverse transcriptase. The following mouse-specific primers were used for the amplification of resistin in WAT: sense AGCTGTGGGACAGGAGCTAA, antisense AGCAAGCTCGACTGCTGG. All mRNA expression values were normalized to cyclophilin and quantified in duplicate, and the averages were used for statistical analysis.

Statistical Analysis

Statview (Abacus) was used for statistical analysis, with a P value <0.05 (two-tailed) considered statistically significant. Data are expressed as means ± SE. Statistical significance was assessed by standard two-tailed Student’s t-tests and ANOVA with Fisher’s post hoc corrections as appropriate. Simple linear and curvilinear bivariate regression analyses were performed to investigate correlations of serum insulin levels and body weights with serum resistin levels in all four cohorts of animals, as well as correlations of serum insulin levels and body weights with resistin WAT mRNA expression in the three cohorts of animals for which mRNA data were available (i.e., lean and/or DIO C57BL/6J mice treated with MTII or CNTFAx15, and TNF-α−/− and WT mice). Bivariate regression analyses were also performed to evaluate for associations between resistin mRNA expression or serum resistin levels and blood glucose levels, with subsequent multivariate regression analyses to adjust for potential confounders, including body weight and insulin levels.

MTII was purchased from Bachem Bioscience (King of Prussia, PA) and dissolved in sterile PBS (30). CNTFAx15 (Axokine) was produced by Regeneron Pharmaceuticals (Tarrytown, NY) and is a variant of the recombinant human CNTF (18, 44). Sera were collected for the analysis of insulin and resistin as previously described (47). All hormones were assayed in duplicate by RIA (rat insulin and mouse resistin; Linco Research Institute, St. Louis, MO).

The sensitivity of the resistin RIA was 0.78 ng/ml, with an intra-assay coefficient of variation at 3.7 ng/ml being 3.6%. Blood glucose levels were measured using a commercially available glucometer (One Touch Profile Blood Glucose Meter; Lifescan, Milpitas, CA) immediately before animals were euthanized. To minimize variability, hormone levels were measured in one assay for all reported studies.
RESULTS

Effect of 24-h MTII Treatment on WAT mRNA Expression and Serum Levels of Resistin in Lean and DIO C57BL/6J Mice

High fat-fed (DIO) C57BL/6J mice exhibited greater initial body weights by ~12 g compared with chow-fed (lean) control mice (Table 1). Both lean and DIO C57BL/6J mice exhibited significant weight loss in response to 24 h of MTII treatment (100 μg qid, P < 0.01 vs. respective PBS-treated groups; Table 1). There was also dose-responsive weight loss with significant decreases after 25 and 100 μg of MTII treatment (qid for 24 h) to lean mice (P < 0.01; Table 1). Moreover, there were significant decreases in food and caloric intake in both lean and DIO mice, with the most significant decreases in caloric intake after 24 h of MTII treatment at 100 μg qid (P < 0.001 vs. respective PBS-treated groups; Table 1). Similar to what was observed with weight loss, there was a dose-responsive decrease in caloric intake in lean mice (P < 0.05 for 25 μg and P < 0.01 for 25 μg; Table 1). There were significant decreases in glucose (P < 0.01 vs. PBS-treated group) and insulin levels (P < 0.05 vs. PBS-treated group) after 24 h of both MTII treatment and pair feeding to DIO mice (Table 1). Although MTII treatment and pair feeding resulted in significant decreases in insulin levels in lean mice (P < 0.05 or <0.001 vs. PBS-treated group for respective lean mice cohorts), glucose levels were not significantly decreased in these mice.

Resistin mRNA expression in WAT was significantly upregulated after 24 h of MTII-treatment to lean mice (P < 0.05 vs. PBS-treated and P < 0.001 vs. pair-fed mice; Fig. 1A), with a dose-specific trend toward increased resistin mRNA expression with increasing MTII doses (79.1 ± 6.6, 90.9 ± 10.5, and 100.3 ± 16.5% for the 5, 25, and 100 μg MTII-treated groups, respectively). DIO mice exhibited an even more significant increase in resistin expression in response to 24 h of MTII treatment (P < 0.0001 vs. PBS-treated and pair-fed DIO mice; Fig. 1A). Similarly, 24 h of pair feeding to DIO mice also resulted in a significant, but less pronounced, increase in resistin expression compared with PBS-treated DIO mice (P < 0.05; Fig. 1A). Although resistin expression was significantly higher in both MTII-treated and pair-fed DIO mice compared with the respective groups of lean mice, resistin expression was similar between PBS-treated lean and DIO mice (Fig. 1A).

In contrast to mRNA expression in WAT, circulating resistin levels exhibited a different pattern of regulation. PBS-treated DIO mice exhibited significantly higher circulating resistin levels compared with all lean mice (P < 0.05 vs. all lean mice; Fig. 1A), whereas MTII-treated and pair-fed DIO mice exhibited only a trend toward higher circulating resistin levels compared with respective lean mice (P = 0.12 for MTII-treated and P = 0.08 for pair-fed mice; Fig. 1A). Moreover, there were no significant differences in resistin levels in response to 24 h of MTII treatment or pair feeding in both lean and DIO mice, indicating that changes in serum resistin levels do not mediate the insulin-sensitizing effects of this drug. Similarly, in a separate experiment, there were no significant differences in serum resistin levels after 24 h of MTII treatment at different doses in lean mice (5 μg: 4.29 ± 0.41; 25 μg: 3.72 ± 0.28; and 100 μg: 4.98 ± 0.82 ng/ml). The differential

Table 1. Effect of high-fat feeding, 24 h of MTII treatment, and 3 (experiment 1) or 7 (experiment 2) days of CNTF treatment (eutanized 4 days after cessation of treatment) on body weight, blood glucose, and serum insulin levels in several mouse models

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial Weight, g</th>
<th>Final Weight, g</th>
<th>Weight Change, g</th>
<th>Cumulative Food Intake, g</th>
<th>Cumulative Caloric Intake, kcal</th>
<th>Glucose, mg/dl</th>
<th>Insulin, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTII Lean vs.</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>DIO C57BL/6J</td>
<td>PBS/chow</td>
<td>32.20 ± 0.89</td>
<td>32.33 ± 1.09</td>
<td>+0.30 ± 0.21</td>
<td>4.09 ± 0.30</td>
<td>14.30 ± 1.00</td>
<td>121.0 ± 4.0</td>
</tr>
<tr>
<td>Pair fed to MTII/chow</td>
<td>32.10 ± 0.76</td>
<td>30.33 ± 0.75</td>
<td>−1.42 ± 0.23**</td>
<td>2.55 ± 0.21***</td>
<td>8.89 ± 0.72**</td>
<td>118.5 ± 2.1</td>
<td>0.79 ± 0.12**</td>
</tr>
<tr>
<td>PBS/high fat</td>
<td>44.07 ± 0.42</td>
<td>42.51 ± 0.72</td>
<td>−1.43 ± 0.23−</td>
<td>2.83 ± 0.3−</td>
<td>12.83 ± 1.6</td>
<td>131.2 ± 3.4</td>
<td>2.90 ± 1.01</td>
</tr>
<tr>
<td>MTII/high fat</td>
<td>43.81 ± 0.76</td>
<td>41.30 ± 0.71</td>
<td>−2.51 ± 0.21*</td>
<td>1.15 ± 0.22**</td>
<td>5.22 ± 1.00**</td>
<td>116.4 ± 3.4</td>
<td>1.24 ± 0.35**</td>
</tr>
<tr>
<td>Pair fed to MTII/high fat</td>
<td>44.27 ± 1.08</td>
<td>42.20 ± 0.94</td>
<td>−2.07 ± 0.19</td>
<td>1.15 ± 0.01***</td>
<td>5.22 ± 0.20**</td>
<td>114.6 ± 1.1</td>
<td>1.35 ± 0.26**</td>
</tr>
<tr>
<td>MTII Lean</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>PBS</td>
<td>24.08 ± 0.63</td>
<td>22.69 ± 0.55</td>
<td>−1.39 ± 0.14</td>
<td>5.16 ± 0.36</td>
<td>18.04 ± 1.21</td>
<td>169.5 ± 8.2</td>
</tr>
<tr>
<td>5 μg qid. MTII</td>
<td>24.03 ± 0.62</td>
<td>22.25 ± 0.61</td>
<td>−1.78 ± 0.13</td>
<td>3.94 ± 0.47</td>
<td>13.74 ± 1.61</td>
<td>173.5 ± 5.5</td>
<td>3.06 ± 0.89</td>
</tr>
<tr>
<td>25 μg qid MTII</td>
<td>24.14 ± 0.59</td>
<td>21.78 ± 0.56</td>
<td>−2.36 ± 0.88***</td>
<td>3.52 ± 0.62*</td>
<td>12.26 ± 2.07**</td>
<td>174.0 ± 5.7</td>
<td>2.04 ± 0.53</td>
</tr>
<tr>
<td>100 μg qid MTII</td>
<td>24.09 ± 0.48</td>
<td>21.34 ± 0.28</td>
<td>−2.76 ± 0.21**</td>
<td>2.46 ± 0.34**</td>
<td>8.88 ± 1.16**</td>
<td>170.0 ± 10.1</td>
<td>0.47 ± 0.09***</td>
</tr>
<tr>
<td>MTII CNT FAx 1</td>
<td>PBS/chow</td>
<td>44.89 ± 1.01</td>
<td>45.42 ± 0.84</td>
<td>+0.53 ± 0.53</td>
<td>29.94 ± 0.74</td>
<td>135.61 ± 3.34</td>
<td>SNA</td>
</tr>
<tr>
<td>Pair fed to 1.0-</td>
<td>45.42 ± 1.20</td>
<td>44.20 ± 1.08</td>
<td>−1.20 ± 0.26**</td>
<td>19.60 ± 0.44**</td>
<td>88.78 ± 2.00**</td>
<td>SNA</td>
<td>2.61 ± 0.36**</td>
</tr>
<tr>
<td>CNT FAx 2</td>
<td>PBS/chow</td>
<td>44.02 ± 2.02</td>
<td>42.70 ± 1.87</td>
<td>−1.32 ± 0.37</td>
<td>37.97 ± 1.40</td>
<td>172.02 ± 6.33</td>
<td>141.2 ± 4.8</td>
</tr>
<tr>
<td>Pair fed to 0.3-</td>
<td>44.60 ± 2.51</td>
<td>36.24 ± 0.09</td>
<td>−8.36 ± 0.60***</td>
<td>13.72 ± 0.01**</td>
<td>62.18 ± 0.03**</td>
<td>96.2 ± 2.1**</td>
<td>2.08 ± 0.43**</td>
</tr>
<tr>
<td>TNPα−/−</td>
<td>PBS/chow</td>
<td>37.02 ± 1.81</td>
<td>45.22 ± 3.40</td>
<td>+8.20 ± 2.19</td>
<td>N/A</td>
<td>N/A</td>
<td>91.0 ± 10.3</td>
</tr>
<tr>
<td>Pair fed to</td>
<td>36.83 ± 1.20</td>
<td>42.03 ± 1.69</td>
<td>+5.20 ± 0.55</td>
<td>N/A</td>
<td>N/A</td>
<td>117.2 ± 13.9</td>
<td>3.10 ± 0.56</td>
</tr>
</tbody>
</table>

Values are means ± SE. MTII, an α-melanocyte-stimulating hormone analog; DIO, diet-induced obesity; CNTF, ciliary neurotrophic factor analog CNTFαx15; TNPα−/−, tumor necrosis factor-α deficient; WT, wild type; SNA, serum not available; N/A, not applicable. To convert glucose from mg/dl to mmol/L, multiply by 5.5 × 10−2; to convert insulin from ng/ml to pmol/l, multiply by 150. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 vs. respective PBS-treated group. #P < 0.05; ##P < 0.01; ###P < 0.001 vs. pair-fed group. †P < 0.01 vs. MTII-treated group on chow. −P < 0.01 vs. PBS-treated group on chow.
regulation of resistin mRNA expression and circulating resistin levels in lean and DIO mice as well as after short-term MTII treatment in lean and DIO mice supports the notion that regulation of resistin expression in WAT does not correlate with circulating resistin levels.

**Effects of 3- and 7-Day CNTFAx15 Treatment on WAT mRNA Expression and Serum Levels of Resistin in DIO C57BL/6J Mice (Euthanized 4 Days After Treatment Cessation)**

Similar to MTII-treated mice, CNTFAx15-treated DIO mice exhibited significant weight loss throughout the 3- and 7-day treatment periods and maintained a lower body weight 4 days after cessation of respective treatments (\(P < 0.0001\) for all CNTFAx15-treated groups vs. respective PBS-treated control groups. \(\#P < 0.05\), \(\##P < 0.01\) vs. respective chow-fed groups. \(*P < 0.0002\) vs. respective pair-fed (PF) groups. \(\daggerP < 0.05\) vs. all chow groups. \(\simP = 0.08\) vs. PF group on chow diet.

Fig. 1. Effect of high-fat feeding, 24 h of α-melanocyte-stimulating hormone analog (MTII) treatment, or 3 days of ciliary neurotrophic factor analog (CNTFAx15) treatment (killed 4 days after cessation of treatment) on white adipose tissue (WAT) resistin mRNA expression and serum resistin levels in several mouse models. TNF-α-/-; tumor necrosis factor-α deficient; \(*P < 0.05\), \(\**P < 0.01\), \(\***P < 0.0001\) vs. respective PBS-treated control groups. \(\#P < 0.05\), \(\##P < 0.01\) vs. respective chow-fed groups. \(*P < 0.0002\) vs. respective pair-fed (PF) groups. \(\daggerP < 0.05\) vs. all chow groups. 

Resistin mRNA expression in DIO mice was significantly downregulated compared with PBS-treated DIO mice euthanized 4 days after cessation of respective treatments (\(P < 0.0001\) for all CNTFAx15-treated groups vs. respective PBS-treated mice; Table 1). Similar results were observed in a separate experiment on DIO mice euthanized 1 day after 7 days of CNTFAx15 treatment (data not shown).

Resistin mRNA expression in DIO mice was significantly downregulated compared with PBS-treated DIO mice euthanized 4 days after 3 days of CNTFAx15 treatment (both 0.1 and 1.0 \(\mu g\) \(\cdot\) \(g\) \(^{-1}\) \(\cdot\) day \(^{-1}\), \(P < 0.05\) and \(P < 0.01\), respectively; Fig. 1B). Despite an apparent dose-dependent decrease in resistin mRNA expression in response to CNTFAx15 treatment, no statistically significant difference was observed between the two active treatment groups. Moreover, resistin mRNA expression remained unchanged in the pair-fed group [\(P < 0.002\) vs. both CNTFAx15-treated groups, \(P = \) not significant (NS) vs. PBS-treated group; Fig. 1B]. A sufficient quantity of mRNA was not available to measure resistin mRNA expression in DIO
mice euthanized 4 days after 7 days of CNTF_{Ax15} treatment, but an effect similar to that reported above for the 3-day CNTF_{Ax15} treatment was also observed in the subcohort of mice killed 1 day after 7 days of CNTF_{Ax15} treatment to DIO mice (CNTF_{Ax15}, 0.1 $\mu$g g^{-1} day^{-1}: 55.5 $\pm$ 13.2%, 0.3 $\mu$g g^{-1} day^{-1}: 38.9 $\pm$ 8.4%, and pair fed: 81.4 $\pm$ 30.8%, compared with PBS-treated controls). In contrast to mRNA expression and similar to the MTII experiment, there were no differences in circulating resistin levels with CNTF_{Ax15} treatment or pair feeding for 3 days (Fig. 1B) or 7 days (data not shown). Overall, consistent with the MTII study, these data support the notion that regulation of resistin expression in WAT does not correlate with circulating resistin levels and do not demonstrate any consistent associations of mRNA expression or serum levels with changes in insulin resistance.

**WAT mRNA Expression and Serum Resistin Levels in TNF-\(\alpha^{−/−}\) and WT Mice**

There were no significant differences between WT and TNF-\(\alpha^{−/−}\) mice in weight gained after 5 wk of high-fat feeding (8.2 $\pm$ 1.9 and 5.2 $\pm$ 0.6 g, respectively), and their final weights were similar to baseline weights in all other obese mouse models studied herein (Table 1). Although there were no significant differences in circulating glucose or insulin levels between TNF-\(\alpha^{−/−}\) and WT mice, glucose levels tended to be elevated in TNF-\(\alpha^{−/−}\) mice, with a significantly lower insulin-to-glucose ratio of 0.03 $\pm$ 0.006 in TNF-\(\alpha^{−/−}\) vs. 0.05 $\pm$ 0.006 in WT mice (\(P < 0.05\)), in agreement with previously published observations (41). Importantly, there were no significant differences in resistin mRNA expression in WAT or circulating resistin levels between TNF-\(\alpha^{−/−}\) and WT mice (Fig. 1C).

**Serum Resistin Levels in UCP1-DTA Mice**

All UCP1-DTA mice were obese (baseline body weight 54.3 $\pm$ 5.12 g). Baseline circulating resistin levels in obese UCP1-DTA mice (7.72 $\pm$ 0.55 ng/ml; \(n = 12\)) were comparable to levels seen in DIO C57BL/6J, obese TNF-\(\alpha^{−/−}\), and obese WT mice, all of which were $\sim$30–50% higher than levels observed in lean C57BL/6J mice.

**Associations of WAT mRNA Expression and Serum Resistin Levels with Body Weight, Serum Insulin Levels, and Blood Glucose Levels**

By simple linear bivariate regression analysis, there was no correlation between resistin WAT mRNA expression levels and both serum insulin levels (std. $\beta = -0.10$, \(P = 0.38\)) and body weight (std. $\beta = -0.04$, \(P = 0.73\)). In contrast, serum resistin levels correlated positively with body weight (std. $\beta = 0.29$, \(P = 0.005\)), but there still was no significant correlation between serum resistin and serum insulin levels (std. $\beta = -0.01$, \(P = 0.90\)). By simple linear bivariate regression analysis, we found no associations between either resistin mRNA expression or serum resistin levels and blood glucose levels (std. $\beta < \pm 0.10$, \(P > 0.05\)). To adjust for potential confounders, we performed multivariate regression analyses introducing the covariates body weight and insulin, first separately and then in combination. With these multivariate adjustments, we again found no association between either resistin mRNA expression or serum resistin levels and glucose levels. We finally performed similar bivariate and multivariate analysis including only the obese mice (DIO C57BL/6J, obese TNF-\(\alpha^{−/−}\), and obese WT mice) and again found no correlation between resistin mRNA expression or serum resistin levels and glycemia.

**DISCUSSION**

In this study, we show that, although there are no differences in WAT resistin mRNA expression between lean chow-fed and high fat-fed DIO C57BL/6J mice, circulating resistin levels are significantly greater in all obese mouse models, including DIO C57BL/6J mice, high fat-fed TNF-\(\alpha^{−/−}\) and WT mice, and BAT-deficient UCP1-DTA mice, compared with lean C57BL/6J mice. Although mice treated with the weight-reducing and insulin-sensitizing compounds MTII and CNTF_{Ax15} have altered resistin WAT mRNA expression, they exhibit no changes in serum resistin levels. Moreover, there are no differences in resistin mRNA expression or in circulating resistin levels in TNF-\(\alpha^{−/−}\) mice compared with WT mice. Finally, neither resistin WAT mRNA expression nor circulating resistin levels are correlated with insulin levels or with glycemia. Thus, by studying several genetic and diet-induced mouse models of obesity and insulin resistance and examining the effect of weight-reducing and insulin-sensitizing drugs, we conclude that there is no correlation between resistin WAT mRNA expression and serum resistin levels, and, importantly, circulating resistin is not correlated with serum insulin or glucose levels.

Prior studies have demonstrated conflicting results with regard to the relationship between adiposity and resistin mRNA expression, i.e., increased resistin expression at the onset of high-fat diet induced obesity in rats (21) vs. decreased mRNA expression in adipose tissue or isolated adipocytes in ob/ob, db/db, tub/tub, KKA\(\beta\), and Zucker (fa/la) genetic models of obesity (16, 19, 25, 26, 43). In contrast to findings regarding adiposity and resistin mRNA expression, we found that DIO C57BL/6J mice have higher circulating resistin levels compared with lean mice and that serum resistin levels correlated positively with body weight in the mouse models studied herein. Moreover, serum resistin levels are similar across all mouse models of obesity, including DIO C57BL/6J mice, high fat-fed obese TNF-\(\alpha^{−/−}\) and WT mice, and BAT-deficient UCP1-DTA mice, which are also obese and develop insulin resistance (11, 23).

These data are in accord with the only previous study that assessed serum levels (by immunoblot) in DIO C57BL/6J mice as well as in ob/ob and db/db mice (38) but did not have the power to statistically evaluate any changes in resistin levels. Whether elevated serum resistin levels in obese mouse models could be at least partly due to higher net secretion of resistin protein (39) or by increased contribution by other tissue cell types, such as monocytes (29), remains to be shown. We also found no differences in resistin WAT mRNA expression or serum levels in high fat-fed obese TNF-\(\alpha^{−/−}\) vs. obese WT mice, suggesting that relative overexpression of TNF-\(\alpha\) does not play a role in the regulation of serum resistin levels in vivo. Prior in vitro studies have shown by Western blot that 3T3-L1 adipocytes treated with TNF-\(\alpha\) have decreased resistin mRNA expression and protein secretion (7, 13, 31, 36), suggesting that TNF-\(\alpha\) is a negative regulator of resistin gene expression, but it is possible that additional factors, which remain to be fully
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elucidated, may be regulating resistin WAT mRNA expression in vivo.

To further investigate the potential role of resistin in obesity, glycemia, insulin resistance, and energy homeostasis, we first assessed cross-sectional associations between serum insulin levels and both resistin WAT mRNA expression and serum resistin levels and then examined the effect of MTII and CNTFAx15, drugs that decrease body weight and improve insulin resistance (30, 37), on resistin WAT mRNA expression and circulating levels. We found no correlation between resistin WAT mRNA expression or serum resistin and insulin levels, further supporting a null effect of circulating resistin on insulin resistance in mice. Prior data have shown increased blood glucose levels and hepatic glucose production after resistin administration (32, 38), and it has recently been reported that mice deficient in resistin have lower blood glucose levels after fasting, secondary to impaired gluconeogenesis (2). Therefore, although our data demonstrate no associations of resistin levels in the normal physiological range with glycemia or insulinemia in several mouse models, we cannot rule out an effect of extreme changes of resistin, i.e., complete absence or supraphysiological levels of resistin, as was the case in previous studies (2, 32).

We also found that both lean and DIO C57BL/6J mice upregulate resistin WAT mRNA expression in response to 24 h of MTII treatment. The effect of MTII in our studies is specific (i.e., not attributed to decreased food intake per se) and may be modified by adiposity. Our data are consistent with prior data showing that treatment with a T2D, another insulin-sensitizing drug, increases resistin expression in both lean and obese rodents, including ob/ob mice and Zucker diabetic fatty rats (8, 43). Treatment of DIO mice with CNTFAx15 (1, 18, 37) results in decreased resistin mRNA expression, an effect that is CNTFAx15 specific. Although the downregulation of resistin mRNA expression in response to CNTFAx15 treatment is in agreement with the previously suggested role of resistin in inducing insulin resistance (12, 13, 36, 38), the upregulation of resistin mRNA expression in response to 24 h of MTII treatment is either in contrast to resistin’s proposed role or indicates a compensatory increase of resistin in response to the insulin-sensitizing effect of MTII. Because MTII may improve insulin resistance by downregulating TNF-α (6, 30), a negative regulator of resistin in vitro (7, 13, 31, 36), we have also explored herein whether a possible compensatory increase of resistin expression could be mediated by altered expression of TNF-α. We found that WAT mRNA expression and serum resistin levels are similar in TNF-α−/− and WT mice, demonstrating that TNF-α is not a regulator of resistin in vivo. In summary, the opposing effects of MTII and CNTFAx15 treatment on resistin mRNA expression, in conjunction with no change in serum resistin levels with either treatment, suggest that the weight-reducing and insulin-sensitizing effects of these drugs may not be mediated by resistin.

In contrast to prior data showing decreased resistin mRNA and protein expression in adipose tissue with fasting (17, 31) and a trend, although not confirmed by statistical analysis, toward decreased serum resistin levels by immunoblot after 48 h of fasting (38), we found no change in serum resistin levels in response to reduced caloric intake (pair feeding) for 3 days. Thus the effect of food restriction on insulin sensitivity does not appear to be mediated by resistin. However, it is possible that the effect of longer periods of food deprivation and/or complete fasting could prove to be different. We (20, 45) previously found that, in humans, serum resistin was not associated with total energy intake, macronutrient intake, or fasting for 48 h.

Human studies on resistin expression provide evidence that resistin may not play a role in insulin resistance, reporting low resistin mRNA levels in human adipocytes (33); no differences in resistin expression in human fat and muscle cells between healthy, insulin-resistant, and type 2 diabetic subjects (28); no correlation between adipocyte gene expression of resistin and insulin resistance (15) or body mass index (33); and no change in resistin expression in human mononuclear cells in response to peroxisome proliferator-activated receptor-γ agonists (33). We (20, 45) and others (46) have demonstrated that there is no association between resistin and insulin resistance, and although there is a positive correlation between serum resistin and adiposity by univariate analysis, this association may not persist after adjustment for potential confounds such as sex (4, 42).

In this study, we have shown that, similar to data in humans, the data in mice presented herein support a null effect of circulating resistin in mediating insulin resistance. Whether resistin may play an important autocrine or paracrine role needs to be elucidated by future studies. In addition, analysis of adipose resistin expression does not correlate with circulating resistin levels and therefore may not be an accurate method for determining resistin’s endocrine role. Future studies investigating in detail the transcriptional and posttranscriptional regulation of resistin, as well as studies on resistin clearance, could elucidate mechanisms underlying the significant discrepancies observed between WAT resistin expression and circulating resistin levels.

REFERENCES


